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Effect of Monensin on the Performance and Nitrogen Utilization of Lactating Dairy Cows Consuming Fresh Forage[1]

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ABSTRACT

We conducted a lactation trial with a fresh forage diet in order to evaluate 1) the effects of monensin on nitrogen metabolism, and 2) the Cornell Net Carbohydrate and Protein System (CNCPS). Thirty Holstein cows in midlactation (eight fitted with ruminal fistulas) were gradually introduced to a fresh forage diet. A concentrate mix based on corn meal was fed before the a.m. and p.m. milking times 0730 and 1730 h, then the fresh forage was fed at 0830 and 1830 h. Fifteen cows each were allocated to a control (no monensin) and a treatment group receiving 350 mg/cow per day of monensin in the p.m. concentrate feeding. A 7-d fecal and urine collection period and a 3-d rumen sampling period were conducted with the fistulated cows. After the lactation study was concluded, the fistulated cows were fed forage regrowth and a 3-d rumen sampling period was repeated. Monensin increased milk production by 1.85 kg. Milk fat and protein concentrations decreased and milk fat and protein yields increased, but the effects were nonsignificant. Monensin did not significantly affect DMI. Ruminal ammonia and the acetate-to-propionate ratio decreased with the addition of monensin in both fed forages. Monensin decreased fecal N output, and increased apparent N digestibility by 5.4%. Because of the decrease in ruminal ammonia and increase in apparent N digestibility, we concluded monensin was sparing amino acids from wasteful rumen degradation with a fresh forage diet. The precision of the CNCPS in predicting performance was high ($r^2 = 0.76$), and the bias was low (overprediction of 3.6%). These results indicate that the CNCPS can be used for dairy cows consuming fresh forage and gives realistic predictions of performance.
(**Key words:** monensin, nitrogen, dairy cows, Cornell Net Carbohydrate and Protein System)

Abbreviation key: CNCPS = Cornell Net Carbohydrate and Protein System, **eNDF** = effective NDF, **ME** = metabolizable energy, **MP** = metabolizable protein, **MUN** = milk urea nitrogen, **PUN** = plasma urea nitrogen.

INTRODUCTION

Dairy cattle have a low efficiency of nitrogen utilization (Castillo et al., 2000), and animal nutritionists have sought mechanisms to enhance digestion and minimize nutrient loss. Nolan (1975) indicated that grazing ruminants can lose as much as 50% of their protein intake as excess ruminal ammonia, and this N is eventually excreted as urinary urea. In recent years, it has become apparent that animal agriculture can have an adverse effect on the environment, the Cornell Net Carbohydrate and Protein System (**CNCPS**) version 4.0 (Fox et al., 2000) has been released for use in developing herd nutrient management plans to minimize excess nutrients on the farm. However, little research has been conducted to evaluate the CNCPS predictions of performance in dairy cattle consuming fresh forages (Kolver et al., 1998).

The carboxylic ionophore monensin has been used to control bloat (Lowe et al., 1990) and to improve average daily gain and feed efficiency in grazing cattle (Potter et al., 1986). Monensin has also improved milk production of lactating grazing cattle (Lowe et al., 1990; Hayes et al., 1996), but responses have not always been statistically significant (Lean et al., 1994). Because the partition and demand for needed nutrients (energy or protein) throughout lactation will vary (Bauman and Currie, 1980), a greater milk production response to monensin supplementation can be expected to occur earlier in lactation. However, even cattle at the end of

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lactation could benefit if feed efficiency increases (Pankhurst et al., 1977).

Monensin decreases methane losses, and the ratio of acetate to propionate (Dinius et al., 1976; Russell and Strobel, 1989). In vivo and in vitro studies indicated that monensin could decrease amino acid deamination and ammonia accumulation (Dinius et al., 1976; Van Nevel and Demeyer, 1977), and later work demonstrated that monensin could inhibit previously unrecognized ruminal bacteria that had very high rates of ammonia production (Chen and Russell, 1989; Russell et al., 1988). The objectives of this experiment were 1) to examine the effects of monensin on the performance and N utilization of lactating cattle consuming a fresh forage diet supplemented with a source of highly degradable rumen carbohydrates, and 2) to evaluate the CNCPS predictions of performance in dairy cows consuming a fresh forage diet.

MATERIALS AND METHODS

This experiment was conducted at the Teaching and Research Center at Cornell University during the summer of 1999. The forage offered was orchardgrass (*Dactylus glomerata* L.) harvested twice daily with a flail chopper and fed immediately after harvest. Thirty multiparous Holstein cows averaging 126 DIM and 560 kg of live weight were blocked in pairs of three based on milk production, live weight, and BCS. Within blocks, cows were randomly assigned to one of two treatments (with and without monensin; M+ and M–, respectively); the cows were fed in individual stalls. Rumen fistulas were previously fitted to eight of the cows in accordance with the Cornell Institutional Animal Care and Use Committee approved procedures.

Before the experiment began, the cows were adjusted from a TMR to the freshly cut orchardgrass forage ration during a transition period of 3 wk. The TMR DMI was measured during 5 d previous to the transition period. The 3-wk transition period was accomplished by incrementally decreasing the amount of TMR offered over the first 2 wk. During the first 4 d of the 2-wk transition period, cows were offered the TMR at the morning feeding (0830 h) at 70% of measured DMI, 50% for the following 5 d, and 25% during the remaining 5 d. The freshly cut orchardgrass was fed ad libitum at 1830 h. Starting on wk 2 of the transition period, a concentrate mix was fed 30 min before the milking times (0730 and 1730 h). During the last week of the diet transition period, the TMR was completely replaced by the freshly cut orchardgrass used for this trial (forage 1). The concentrate mix feeding preceded the milking times, and, therefore, forage and grain were fed separately to mimic the feeding pattern of a grazing animal. During the 3-wk transition period, the average milk production dropped 2.4 kg. Monensin was fed at half of the final dose (175 mg/cow per day) in the p.m. concentrate feeding beginning on d 2 of wk 3 of the diet transition period. The full dose (350 mg/cow per day) was introduced 4 d later. The monensin lactation trial was initiated 3 d after the full dose was introduced and was terminated after 17 d because of a lack of forage due to dry weather. The monensin lactation effects were analyzed based on a 17-d period (monensin trial) starting 3 d after starting the full dose.

The amount of minerals and vitamins fed was based on NRC (1989) recommendations. The concentrate mix fed to the cows consisted of 86.9% cornmeal, 6% molasses, 2.45% $CaCO_3$, 1.53% $CaHPO_4$, 1.3% MgO, 0.96% NaCl, 0.61% $CaSO_4$, 0.12% Se premix (0.06% Se), 0.05% trace mineral premix (Round House Mill, Cortland, NY), and (per kilogram of concentrate DM) 8900 IU of vitamin A, 2700 IU of vitamin D, and 34 IU of vitamin E. The cows received 3.2 kg of this concentrate mix before the a.m. and p.m. milking times (0730 and 1730 h). The freshly cut orchardgrass was fed ad libitum after the milking times (0830 and 1830 h). The amount of concentrate fed was calculated to be approximately 30% of the total DMI to test the CNCPS predictions of performance with a fresh forage-based diet. A 7-d fecal-urine collection period was conducted with the eight ruminally fistulated cows that were kept in metabolism stalls. Ruminal fluid was collected on 3 different days (d 15 to 17 of the monensin trial). After the lactation study was concluded, the fistulated cows were fed a fresh regrowth forage (forage 2) for an additional 12 d. Following those 12 d, a 3-d ruminal sampling period was repeated.

Model Evaluation

The CNCPS model validation was conducted with the cows fed the control diet during the 17 d of the monensin trial plus the 3 preceding days. In the CNCPS 4.0 (Fox et al., 2000) feed physical characteristics are described as effective NDF (**eNDF**). The eNDF value is defined as the percentage of the NDF retained on a 1.18-mm screen; measurement of fresh forage according to this definition will overestimate eNDF (Kolver et al., 1998). Within the structure of the CNCPS, the eNDF of a feed is used to predict ruminal pH and to adjust passage rate. Therefore, the eNDF value of the fresh forage was the value required for the predicted rumen pH of the average of the control cows to match the mean measured rumen pH.

Sample Collection and Analysis

Milk production was recorded daily at 0800 and 1800 h during the 21-d transition period and the following

20 d of the trial. During the trial, milk samples were collected at the a.m. and p.m. milkings. Samples were preserved with 2-bromo-2-nitropropane-1, 3-diol and were analyzed for fat, protein, milk urea nitrogen (**MUN**), and SCC at the New York DHIA milk testing laboratory (infrared analysis; Foss 605B Milko-Scan; Foss Electric, Hillerød, Denmark). On d 5, 6, 11, 12, 16, and 17 of the monensin trial, a.m. and p.m. milk subsamples were also analyzed for MUN with a manual urease/Berthelot determination (Sigma urea nitrogen procedure no. 640, Sigma Diagnostic, St. Louis, MO). On d 5 to 9 and 11 to 17 of the monensin trial, blood samples were drawn from the coccygeal vein 3 h after the a.m. milking. Samples were immediately placed on ice and centrifuged at $3000 \times g$ for 15 min at 4°C; then, plasma was collected and stored at –20°C. Plasma was analyzed for plasma urea N (**PUN**) (Sigma, urea nitrogen procedure no. 640, Sigma Diagnostic).

Forage and grain intakes were measured daily starting on wk 3 of the transition period until the end of the trial by weighing a.m. and p.m. feed offered and refused. The amount of orchardgrass offered was adjusted for 15% orts; a.m. and p.m. microwave DM checks taken daily were used for this calculation. The a.m. and p.m. forage samples were collected during the 20 d of the model validation trial. A first subsample was dried at 60°C in a forced-air oven during 48 h for DM determination. A second subsample of the forage offered was frozen in liquid nitrogen, stored at –20°C, and subsequently freeze-dried. Forage samples were ground to pass a 1-mm screen in a Wiley mill (model 4, Arthur H. Thomas Co. Philadelphia, PA). Samples were composited within a.m. and p.m. for each week of the 20-d period (wk 1 was a 6-d period, and wk 2 and 3 were both 7-d periods). Samples of the concentrate offered for each week were stored at 4°C, ground to pass a 1-mm screen as described before, and were then composited before analysis. All feed samples were analyzed for DM, Kjeldahl N using boric acid (Pierce and Haenisch, 1947), NDF, and ADF using sodium sulfite for NDF, and acid detergent lignin (Van Soest et al., 1991). All protein fractions, buffer-soluble protein, NPN, ADIN, and neutral-detergent insoluble nitrogen were determined according to the procedure of Licitra et al. (1996). Ash and ether extract were analyzed according to the AOAC (1990). The orchardgrass and grain mix degradation kinetics were determined with the gas production procedure as described by Pell and Schofield (1993).

Collection period. During the fecal-urine collection period (7 d from d 11 to 17, of the monensin trial), forage and ort samples were collected for each cow. Samples were dried at 60°C in a forced-air oven during 48 h for DM determination, subsequently ground to pass a 1-mm screen in a Wiley mill, and composited by volume across the 7-d period. Samples were analyzed for NDF and Kjeldahl N as described before.

Urine was collected from the eight fistulated cows via a Foley catheter. The day before the catheters were placed, urine samples were collected by eliciting micturition by manual stimulation of the vulva in order to assess the amount of acid needed to bring urinary pH to approximately 3. Urine was collected in buckets with 400 ml of 20% H_2SO_4, a new bucket was allocated after each milking. Each morning at the end of a 24-h period, the two daily buckets were mixed, and a daily sample (1% of volume) was collected, and stored at –20°C. Samples were thawed, subsequently composited within cow, and analyzed for Kjeldahl N as described before.

Feces were collected every 24 h. A daily sample (3% of volume) was collected and stored at –20°C. After the experiment was completed, fecal samples were thawed, composited within cow, and analyzed for DM, NDF, and Kjeldahl N (on wet samples) as previously described.

The milk N secretion calculation was obtained from DHIA milk protein data.

Ruminal fermentation. Ruminal fluid from the eight fistulated cows were sampled on d 15, 16, and 17 of the monensin lactation trial every 3.5 h from 0730 to 0015 h. The interval between samples 3 and 4 was shortened to 3 h to sample the rumen before the afternoon grain feeding. The 3-d sampling schedule was repeated with forage 2. Ruminal fluid was collected by suction for at least five locations in the rumen. The samples were composited (500 ml total) and strained through four layers of cheesecloth. A subsample (50 ml) was chilled to 5°C, transported to the laboratory, and centrifuged at $500 \times g$ (5 min, 5°C) to remove feed particles and protozoa. The sample was then centrifuged at $10{,}000 \times g$ (15 min, 5°C) to remove bacteria. A portion of the clarified ruminal fluid (10 ml) was frozen for ammonia and VFA analyses. The remaining clarified ruminal fluid was placed in a 39°C water bath and purged slowly with CO_2 for 15 min. The pH of the clarified and CO_2 equilibrated ruminal fluid was determined with a combination electrode. Preliminary experiments indicated that these pH measurements were identical to those taken on ruminal fluid that was immediately removed from the cow. Ammonia in cell-free ruminal fluid was measured by the colorimetric method of Chaney and Marbach (1962). Ruminal VFA were quantified by HPLC (Beckman model 334 liquid chromatograph, model 156 refractive index detector, model 421 CRT data controller, CR1A integrator, Bio-Rad HPX-87H organic acid column, 20-μL loop, 0.013 N H_2SO_4, 0.5 ml/min, 50°C).

Statistical Methods

Milk yield data. Milk production data from the monensin lactation trial were analyzed based on residuals

from a test-day model as described by Van Amburgh et al. (1997). After residuals were obtained, they were analyzed using PROC GLM of SAS (1999) according to the model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk},$$

where:

Y_{ijk} = all dependent variables,
μ = overall mean,
α_i = treatment effect,
β_j = block effect,
$(\alpha\beta_{ij})$ = interaction between treatment and block effects, and
e_{ijk} = random error term.

The interaction $(\alpha\beta)_{ij}$ was used as the error term to test the factor α_i. Differences between test-day model residuals were considered to be the treatment differences. The other lactation variables (MUN, PUN, and forage and concentrate DMI) were analyzed using the same statistical model based on the observed data.

Rumen data. A repeated measures design was used to test sampling time and its interaction with treatment for each ruminal variable. We analyzed the orthogonal components of the Mauchly's sphericity criterion to test the Huynh-Feldt assumption; same variance of the treatment difference for all possible pairs at different sampling time. If it failed to reject the null hypothesis ($P > 0.05$), we analyzed the data as a split-unit design (Kuehl, 2000); otherwise, we used the adjusted F (Greenhouse-Geisser Epsilon) to test the interaction between time and treatment. We assumed compound symmetry, and equal correlation among repeated measures, in this analysis. The statistical model is described below:

$$Y_{ijkml} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + c(a)_{k(i)} + bc(a)_{jk(i)} + d_l + e1_{ijkl} + \gamma_m + \alpha\gamma_{im} + \beta\gamma_{im} + \alpha\beta\gamma_{ijmt} + c(a)g_{k(i)m} + bc(a)g_{jk(i)m} + dg_{lm} + e2_{ijklm},$$

where:

Y_{ijkml} = all dependent variables;
μ = overall mean;
α_i = monensin effect;
β_j = forage effect;
$\alpha\beta_{ij}$ = interaction between monensin and forage effects;
$c(a)_{k(i)}$ = cow within monensin as a random factor;
$bc(a)_{jk(i)}$ = interaction between forage and $c(a)_{k(i)}$, as a random factor;
d_l = days as blocking factor;
$e1_{ijkl}$ = random error term 1 for the plot unit effects;
γ_m = sampling time effect;
$\alpha\gamma_{im}$ = interaction between monensin and sampling time effects;
$\beta\gamma_{im}$ = interaction between forage and sampling time effects;
$\alpha\beta\gamma_{ijmt}$ = interaction among monensin, forage, and sampling time effects;
$c(a)g_{k(i)m}$ = interaction between $c(a)_{k(i)}$ and sampling time effects as a random factor;
$bc(a)g_{jk(i)m}$ = interaction among forage, $c(a)_{k(i)}$, and sampling time effects as a random factor;
dg_{lm} = interaction between days and sampling time effects as a random factor; and
$e2_{ijklm}$ = random error term 2 for time and time interaction effects.

The term $c(a)_{k(i)}$ was the error term to check α_i effects, and the $bc(a)_{jk(i)}$ was the error term for β_j and $\alpha\beta_{ij}$ interaction.

Ruminal pH versus VFA. Rumen pH was regressed against total VFA (m*M*) concentration using categorical predictor variables (M– and M+ and forages 1 and 2) to test for different intercepts and slopes among treatment effects. The statistical model is described below:

$$Y_i = \beta_0 + \beta_1 X_{i1} + \beta_2 X_{i2} + \beta_3 X_{i3} + \beta_4 X_{i4} + \beta_5 X_{i5} + \beta_6 X_{i6} + \beta_7 X_{i7} + \varepsilon_i,$$

where:

Y_i = rumen pH,
X_1 = 1 (indicator M–, and Forage 1), 0 otherwise;
X_2 = 1 (indicator M–, and Forage 2), 0 otherwise;
X_3 = 1 (indicator M+, and Forage 1), 0 otherwise;
X_4 = VFA (m*M*);
X_5 = interaction between X_1 and X_4;
X_6 = interaction between X_2 and X_4;
X_7 = interaction between X_3 and X_4; and
ε_i = random error term.

The 7-d fecal-urine collection period was analyzed as a CRD model:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

where:

Y_{ij} = all dependent variables,
μ = overall mean,

α_i = monensin effect, and
ε_{ij} = random error term.

In all statistical models, studentized residual plots were used to check for outliers and homogeneity of variance. Normality was evaluated using the distribution plot of the standardized residuals (Neter et al., 1996).

Carbohydrate digestion rates. The parameters of the orchardgrass and grain mix sample gas production curves were obtained by fitting the following nonlinear equation (Mertens and Loften, 1980):

$$V = V_F e^{-k(t-L)}$$

where:

V = volume of gas produced at time t,
V_F = volume of gas from complete substrate digestion,
k = digestion rate constant, and
L = discrete lag time.

The parameters of the equations were obtained by the NLIN procedure in SAS (1999). The data used in this curve-fitting included observations from the fermentation of the unfractionated, and the ND-insoluble fractions, for each of the orchardgrass and grain mix samples.

Model evaluation. The objective of a model evaluation is to determine the precision (repeatability of a prediction), and accuracy (the closeness with which a prediction approaches its true value) of the model subject to investigation (Cochran and Cox, 1957). Accuracy, the most important characteristic of a model, can be assessed by computing the mean bias (Cochran and Cox, 1957):

$$\text{Mean bias} = \frac{1}{n}\sum_{i=1}^{n}(\text{predicted}_i - \text{observed}_i)$$

A regression analysis of model predictions was conducted by regressing the observed milk production against the model predicted milk production [first limiting metabolizable energy (**ME**) or metabolizable protein (**MP**) allowable milk (Kohn et al., 1998)], as described by (Mayer and Butler, 1993). The slope of the regression when forced through the origin minus one has been referred as the model bias. Because of the ambiguity of testing whether the slope of the regression differs significantly from 1 when there is much scatter around the line (Mitchell, 1997), the model bias was calculated by dividing the mean of the Y-variate minus the mean of the X-variate by the mean of the X-variate (Tedeschi et al., 2000). The statistical measures of model precision we used were the regression r^2, standard error, and the residual plot, which is the studentized residuals plotted against regression predicted (Mayer and Butler, 1993). Residual plots were analyzed for outliers and systematic bias (Neter et al., 1996). Regression parameters were estimated by PROC REG, and the statistical comparison between observed and predicted values was performed using the two-sample *t*-test (SAS, 1999).

RESULTS

When lactating dairy cattle were fed a fresh forage with cornmeal as an energy supplement, the CNCPS predicted milk production was highly correlated with the observed milk production (Figure 1A) and the bias was low (Figure 1B). The eNDF value of the fresh forage (where the predicted rumen pH matched the mean measured pH) was 43%. As the lactation trial progressed, forage NDF and lignin as a percentage of the NDF increased and available NDF digestion rate and the in vitro NDF digestibility decreased (Table 1). Forage CP also decreased during the lactation trial, and the regrowth (forage 2) was higher in CP.

The mean milk production for the preliminary period and the milk production of the 17-d monensin trial period are shown in Figure 2. The milk production response to monensin was 1.85 kg (6.5%) ($P < 0.05$; Table 2). The treated cows had a 0.12 percentage unit decreased in fat content, and a 0.06 percentage unit decreased in protein content. Monensin resulted in a 4.6% increased in fat yield, and protein yield increased by 4.7%. Although these effects on milk composition were nonsignificant, the trends are in agreement with the increase in milk production. There were no MUN treatment differences for either DHI or the colorimetric method. However, the difference between methods was significant; the colorimetric method was 2.7 percentage units higher than DHI reported values ($P < 0.001$). PUN levels taken 3 h after the a.m. milking did not differ between treatments.

Fresh forage and concentrate DMI were not different between control and monensin cows (Table 2). Total DMI as a percentage of BW and NDF intake as a percentage of BW averaged 3.67 and 1.51%, respectively. Of the total daily fresh forage DM consumed, 41% was from the morning feeding and 59% was from the afternoon feeding. This consumption pattern was not influenced by monensin.

Monensin had no effect on ruminal pH or total VFA (Table 3). When pH was regressed against total VFA, neither monensin nor forage treatments had different intercepts and slopes (Figure 3). Increased total VFA

caused a decrease in ruminal pH ($r^2 = 0.6$). Monensin dependent decreases ($P < 0.05$) in the acetate-to-propionate ratio were caused by an increase ($P = 0.17$) in propionate and decrease ($P = 0.10$) in acetate (Table 3). Monensin decreased the acetate-to-propionate ratio from 3.8 to 3.1 for forage 1 (used for the lactation trial, $P < 0.05$), and from 4.8 to 3.7 for forage 2 (regrowth, $P < 0.01$) (Figure 4A). Forage 1 had a lower ($P < 0.01$) acetate-to-propionate ratio than forage 2. The lower ($P < 0.01$) acetate-to-propionate ratio for forage 1 was caused by a lower ($P = 0.12$) acetate and a higher ($P = 0.24$) propionate compared with forage 2. Butyrate concentrations increased by 10% ($P < 0.01$) when forage 2 was fed compared with forage 1.

When forage 2 was fed, rumen ammonia increased ($P < 0.001$) 2.4 times compared with forage 1 (Table 3). Monensin decreased rumen ammonia from 6.07 m*M* to 5.03 m*M* ($P = 0.30$, Table 3). There was no interaction between monensin and forage ($P = 0.68$), and within-forage treatment (Figure 4B) monensin decreased rumen ammonia from 3.7 to 2.8 m*M* for forage 1 ($P < 0.08$), and from 8.4 to 7.2 m*M* for forage 2 ($P < 0.05$).

Nitrogen intake, partitioning, and digestibilities are shown in Table 4. Nitrogen intake was not different between control and monensin cows. Fecal nitrogen output was lower ($P < 0.05$) for monensin cows. Monensin treatment increased ($P < 0.07$) the apparent nitrogen digestibility by 5.4% compared with control cows. Urinary N output was not different, but the variance was high.

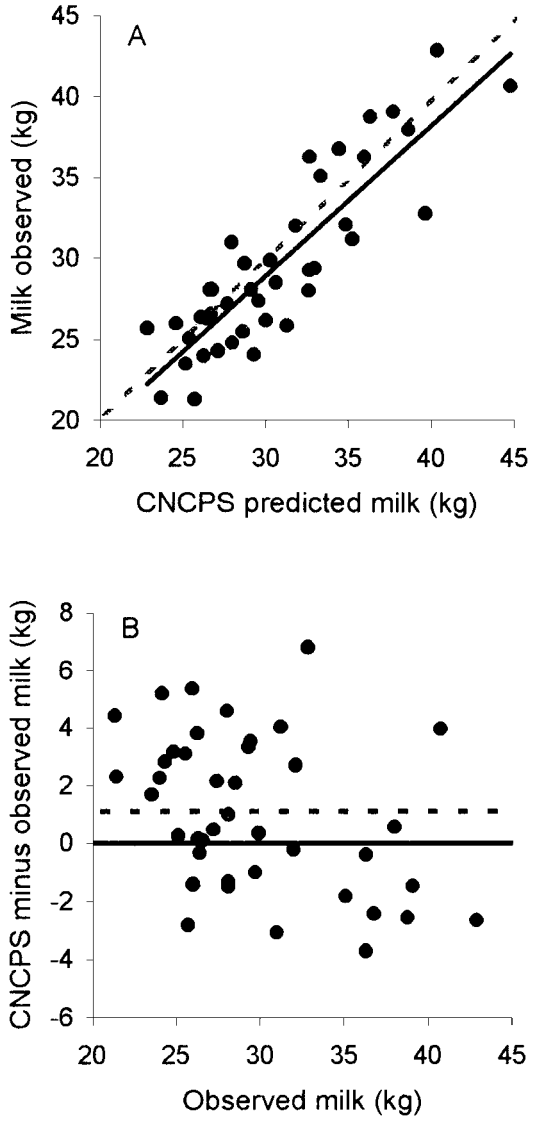

Figure 1. Prediction of milk production by the Cornell Net Carbohydrate and Protein System (CNCPS). (A) Relationship between observed (o) milk (M) and CNCPS-predicted (p) milk. The regression is $M_o = 0.87 + 0.94 \times M_p$, $r^2 = 0.76$, SE = 0.41. The line Y = X (dashed line) represents agreement between observed and predicted milk production. (B) Deviation (CNCPS-predicted minus observed milk) vs observed milk. Solid line represents no bias or error. Dashed line represents the mean bias, 1.1 kg of milk (over prediction bias of 3.6%, $P < 0.05$). Data shown are for individual weeks of observations on individual control cows.

DISCUSSION

Identifying the nutritional constraints of a diet, and minimizing nutrient loss from the farm are the main objectives of the CNCPS. Lush spring pastures often have an abundance of protein, and, under these conditions, ME can be the first limiting nutrient (Waghorn and Barry, 1987). However, the ratio of ME to MP allowable milk can be affected by production level, ruminally degradable carbohydrate supplements, and changes in pasture quality.

Kolver et al. (1998) found that the CNCPS realistically predicts performance when cows are fed high quality pastures limited by the supply of ME and suggested that certain amino acids may limit milk production when more than 20% of the diet consists of a grain supplement. In the current study, the precision of predicted versus observed milk production was high ($r^2 = 0.76$), the bias was low (over-prediction of 3.6%), and the CNCPS indicated that our diets had a lower MP allowable milk than ME allowable milk. The eNDF value (percentage of NDF effective in stimulating chewing and salivation, rumination, and rumen motility; Mertens, 1997) of the fresh forage is in agreement with previous reported values for fresh forages (Kolver et al., 1998). The diets were supplemented with a concentrate mix (87% corn meal). However, the rumen nitrogen balance was positive, and it appeared that the ruminal bacteria did not have enough energy to utilize all of the ruminally degraded protein. Based on these results, it appeared that monensin-dependent increases in milk

Table 1. Chemical composition, and gas production kinetics of neutral detergent (ND)-soluble and digestible NDF fracations of the fresh forage and concentrate mix.

Item	Forage 1[1]			Forage 2[1]	Concentrate mix
	1	2	3		
DM, %	26.3	30.2	32.9	32.4	88.4
NDF, % of DM	49.9	52.3	53.1	50.3	11.2
Lignin, % of NDF	5.5	7.1	7.7	7.9	3.8
CP, % of DM	17.6	17.3	16.4	21.3	8.6
Sol P, % of CP	35.4	36.6	40.3	32.7	10.4
NPN, % of Sol P	38.6	42.1	50.1	38.4	65.0
NDFIP, % of CP	16.0	15.7	16.8	24.9	21.4
ADFIP, % of CP	2.3	2.6	2.7	3.2	6.1
Fat, % of DM	4.3	4.4	4.9	5.5	3.3
Ash, % of DM	5.3	5.1	5.0	5.5	7.3
Ca, % of DM	0.37	0.38	0.37	0.60	1.48
P, % of DM	0.41	0.40	0.33	0.35	0.68
Carbohydrate degradation rates, % h					
ND-soluble (A + B1)	19.5	17.3	25.2	13.2	15.3
Digestible NDF (B2)	6.5	6.2	5.9	6.9	11.0
IVNDFD,[2] % NDF	76.8	68.9	61.5	67.9	84.7

[1]Values are means of a.m. and p.m. weekly composite samples.

[2]IVNDFD = In vitro NDF digestibility.

production could be driven by changes in MP as well as ME.

Monensin increased ($P < 0.05$) the milk production of our cattle, and the magnitude of this increase (6.5%) is in agreement with previously reported data (Van Der Werf et al., 1998). The numerically increased fat and protein yield are also in agreement with previous reported data (Beckett et al., 1998). Monensin decreased the ruminal acetate-to-propionate ratio approximately 1.25-fold. Cattle fed grain have higher propionate-to-acetate ratios than those fed forage, but Ramanzin et al. (1997) noted that even lactating cattle fed an abundance of grain had a significant increase in propionate when monensin was fed. When forage-to-concentrate ratio was 50:50, monensin increased the percentage of propionate to a greater extent than when the forage-to-concentrate ratio was 70:30. However, when Van Maanen et al. (1978) measured the propionate production on two diets with different forage-to-concentrate ratios (70:30 and 20:80), no interaction between monensin and diet was found. Based on these results, the authors concluded that molar percentages do not accurately indicate changes in propionate production, and this conclusion was supported by the work of Roger and Davis (1982).

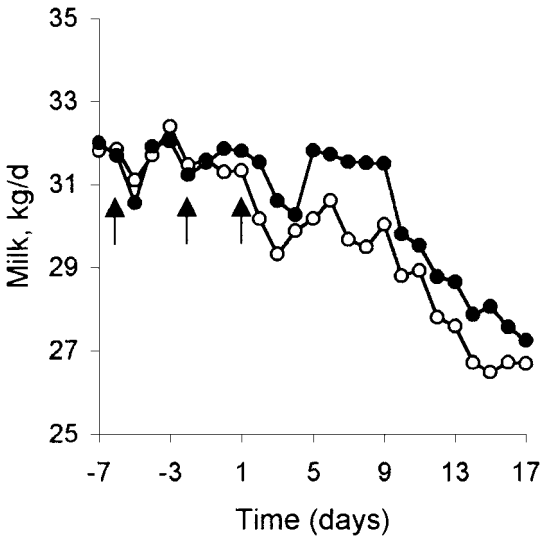

Figure 2. Actual milk production for control (○) and monensin-treated (●) cows. The first arrow represents when monensin was introduced at half of the final dose (175 mg/cow per day); the second arrow indicates when the full dose was initiated (350 mg/cow per d). The third arrow indicates the beginning of the 17-d monensin trial period.

In feedlot cattle, the response to monensin has typically been explained by an increase in energy utilization (Wedegaertner and Johnson, 1983). However, energy and protein are related, and in lactating cattle increases in energy supply have given increases in protein utilization (Mackle et al., 1999). Lana et al. (1997) noted that monensin improved average daily gain of Holstein steers fed either soybean meal or urea, but the impact of monensin on feed and nitrogen utilizations were greater for soybean meal than for urea. Based on these results, the authors concluded that monensin spared amino acids.

Hayes et al. (1996) reported that monensin-treated cows had higher blood urea N, and they suggested that

Table 2. Least squares means for lactation variables of the control, and monensin treated cows (difference from control).

				Significance[a]		
	Control	Monensin	SE	M	T	T × M
Milk production, kg/d	28.49	+1.85[b]	0.41	*	0.43	0.84
Fat, %	3.42	–0.12[c]	0.09	0.42	**	0.28
Protein, %	2.89	–0.06[c]	0.06	0.50	**	0.29
Fat yield, g/d	974	+45[b]	0.04	0.48	0.79	0.73
Protein yield, g/d	823	+39[b]	0.02	0.36	0.58	0.70
MUN DHI, mg/dl	12.07	+0.31[c]	0.53	0.71	**	0.80
MUN (colorimetric), mg/dl	14.09	+1.69[c]	0.55	0.10	. . .	. . .
PUN, mg/dl	14.88	–0.21[c]	0.69	0.84	. . .	. . .
Total DMI, kg/d	20.83	–0.33[c]	0.45	0.63	**	0.11
Pasture intake, kg/d	15.47	–0.57[c]	0.35	0.32	**	0.20
Concentrate intake, kg/d	5.37	+0.34[c]	0.16	0.20	**	0.40

[a]M = Monensin; T = time, as day number.

[b]Least square means difference based on residuals from TDM.

[c]Least square means difference based on observed data.

*$P < 0.05$.

**$P < 0.001$.

monensin increased the escape of undegraded protein from the rumen. We did not measure protein escaping the rumen, but we were able to demonstrate a decrease in ruminal ammonia. Previous work by Yang and Russell (1993) indicated that monensin-dependent decreases in ruminal ammonia could be correlated with an increase in microbial protein, but other workers indicated that monensin decreased ruminal ammonia, and increased dietary but not microbial protein flow from the rumen (Haïmoud et al., 1996). Decreases in ruminal ammonia, and increases in dietary protein flow from the rumen have also been associated with decreases in microbial protein flow from the rumen (Muntifering et al., 1981).

Periparturient dairy cows might go into negative protein balance until d 28 of lactation (Bell et al., 2000). When Plaizier et al. (2000) supplemented transition dairy cows with monensin, there was a numerical decrease in rumen ammonia, an improvement in apparent N digestibility, and a reduction in the negative N balance, and monensin appeared to spare amino acids from wasteful degradation in the rumen. Because the fecal N of our treated cattle was lower than the controls, it appeared that monensin was increasing N digestibility, but we did not determine the ratio of microbial to feed protein leaving the rumen. Feed CP is usually more digestible than bacterial CP (Van Soest, 1994), and amino acid digestion in the small intestine can increase due to monensin supplementation (Haïmoud et al., 1995). An increase in the ratio of dietary escape protein to microbial protein flow from the rumen could cause an overall improvement in N digestibility. Increased protein flow to the small intestine will up-regulate the amino acid uptake capacity of the small intestine, re-

Table 3. Least squares means for monensin and forage rumen effects.

	Treatment								Significance[a] *P* value				
	Monensin				Forage								
Measurement	0 mg/d	350 mg/d	SE	(*P* value)	1	2	SE	(*P* value)	M × F	T	T × M	T × F	T × M × F
pH	6.15	6.22	0.04	(0.28)	6.16	6.20	0.02	(0.23)	0.50	***	0.27	**	0.23
Total VFA, m*M*	102.72	99.35	4.37	(0.60)	100.50	101.56	1.92	(0.71)	0.20	***	0.84	**	0.21
Acetate, m*M*	72.64	67.11	2.05	(0.10)	68.76	70.99	0.87	(0.12)	0.21	***	0.92	**	0.06
Propionate, m*M*	17.45	21.49	1.82	(0.17)	20.60	18.34	1.21	(0.24)	0.27	***	*	***	0.63
Butyrate, m*M*	12.62	10.80	0.94	(0.22)	11.14	12.28	0.18	(**)	0.57	***	0.06	***	0.19
Acetate:Propionate	4.28	3.39	0.18	(*)	3.45	4.23	0.10	(**)	0.16	***	0.07	*	0.52
Ammonia, m*M*	6.07	5.03	0.64	(0.30)	3.29	7.82	0.21	(***)	0.68	***	*	***	0.06

[a]M = monensin; F = forage; T = sampling time.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

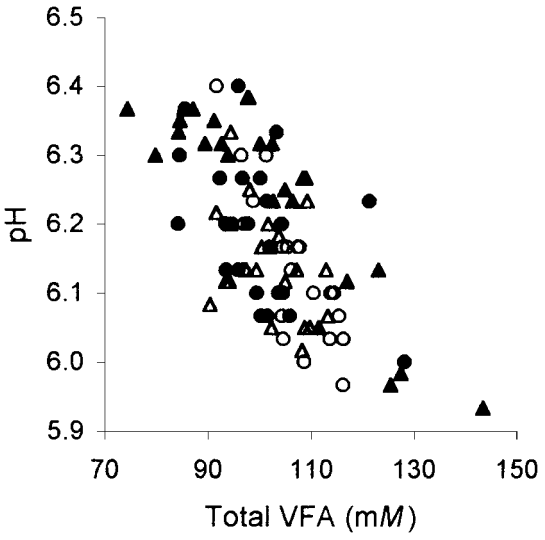

Figure 3. The relationship between ruminal VFA concentration and ruminal pH. $r^2 = 0.60$. (○) M–, and forage 1; (●) M+, and forage 1; (△) M–, and forage 2; and (▲) M+, and forage 2.

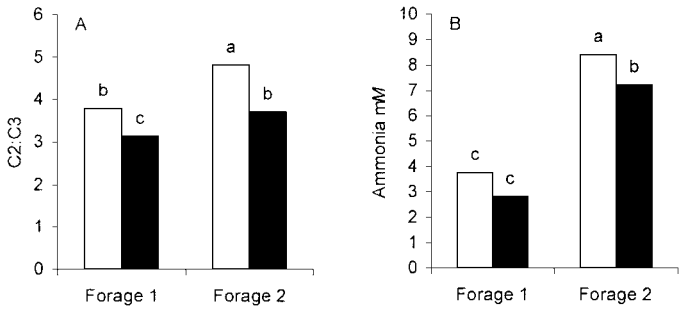

Figure 4. Effect of monensin and forage quality on the acetate-to-propionate ratio (A), and rumen ammonia (B). (□) M–, and (■) M+. Bars with different superscripts differ ($P < 0.05$) within and between forages.

sulting in a greater extraction of amino acids from the intestinal lumen (Stevens, 1992).

Urinary N output of monensin-treated cattle was higher than the controls, but this effect was not statistically significant. However, monensin significantly decreased fecal N excretion. Based on these results, it appeared that monensin altered the pattern of N excretion. Fecal N is made up of bacterial CP, undigested feed materials, and endogenous secretions, and urea can pass from the blood into the gut to drive additional microbial protein synthesis (Kennedy and Milligan, 1978). The impact of monensin on microbial growth in the lower gut has not been well defined, but work with pigs indicated that salinomycin, another ionophore, depressed microbial N synthesis in the intestinal tract (DeWilde, 1984). If monensin had a similar impact on cattle, it is conceivable that monensin could decrease urea flux from the blood to the gut (by depressing lower gut microbial growth) and decrease fecal N excretion.

Other workers noted that monensin significantly increased ruminal pH in periparturient dairy cows fed a TMR diet (Green et al., 1999), and increases in rumen pH have been explained by lower lactate concentrations (Nagaraja et al., 1981). In our study with fresh orchardgrass, monensin did not significantly increase ruminal pH. However, ruminal pH values were negatively correlated ($r^2 = 0.60$) with VFA concentration (Figure 3). Lactate was never detected, and these results support the idea that ruminal pH can decline as a function of VFA concentration (Briggs et al., 1957) even if lactate is not present.

Rainfall was particularly low during the time this trial was conducted, and the lack of rainfall and warm temperatures accelerated the maturation of forage 1, resulting in CP values that were lower than the regrowth (forage 2). Therefore, ruminal ammonia concentration was higher for forage 2. Monensin only caused a statistically significant decrease in rumen ammonia when forage 2 was fed (Figure 4B). In transition dairy cows, monensin numerically decreased rumen ammonia, improved apparent N digestibility, and improved

Table 4. Digestibility and efficiency of N utilization by control and monensin treated cows.

	Control	Monensin	SE	$P<$
DMI, kg/d	19.9	19.1	0.80	0.52
Total N intake, g/d	452.2	436.0	21.38	0.61
From fresh forage, g/d	370.7	354.3	24.13	0.65
From concentrate, g/d	81.5	81.7	4.40	0.97
Fecal N output, g/d	156.9	135.8	5.19	0.03
Urine N output, g/d	141.6	150.7	14.91	0.67
Milk N output, g/d	105.6	111.0	8.49	0.66
Digestibility, %				
Apparent TTD[1]	68.6	69.6	0.90	0.45
Apparent N	65.2	68.7	1.10	0.07
NDF %	56.0	56.9	1.07	0.54

[1]Total tract digestibility.

the nitrogen balance during the periparturient period (Plaizier et al., 2000). These results support the idea that monensin spared amino acids from wasteful degradation in the rumen.

The question then arises, did monensin have a nutritionally significant impact on the protein utilization of our cattle? The monensin-dependent decreases in ruminal ammonia concentration and fecal N excretion indicated that the treated cattle might have had a greater supply of intestinal amino acids. When MP is the first limiting nutrient, it is likely that a positive impact on milk production will come from greater amounts of undegraded feed protein flow from the rumen.

CONCLUSIONS

Monensin has the potential to increase the efficiency of N utilization in dairy cows fed fresh forage and to decrease fecal N excretion. Because of the decrease in ruminal ammonia and increase in apparent N digestibility, the results of our trial suggest that monensin spared amino acids from wasteful degradation in the rumen.

The results of this study demonstrate that the CNCPS can be used to formulate diets for dairy cows consuming fresh forages, and gives realistic predictions of performance under these conditions.

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